“Applications of microfluidics in biology and medicine”

PhD thesis by mgr inż. Witold Postek

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Date of compiling the summary: 6. June 2019

Summary

The aim of this doctoral dissertation was to develop a high-throughput method of screening and analyzing antibiotic resistance at the level of single bacterial cells. The research conducted by the author can be divided into the construction of the microfluidic platform (A, B, C) and the application of this platform to studies of antibiotic resistance at the single-cell level (D).

A. Precise and accurate dilution of samples in microliter droplets

In this part of the dissertation the goal was to develop a microfluidic device that would generate a series of dilutions of a sample so that the dilutions would be as reproducible as possible, and so that it would be possible to add a precisely metered portion of another sample to each of the dilution the series.

The problem of imperfect fabrication of numerous copies of channel geometries for metering of droplets needed to be addressed. The developed and here described solution allows for achieving a constant dilution factor with high precision and accuracy by metering all the droplets participating in the dilution process in a single metering trap. An add-on module was developed that allowed for addition of precisely metered droplets with another sample to each droplet in the dilution series.

This part of work is described in the chapters 3.1. and 4.1.

B. Passive emulsification of multiple aqueous microliter plugs

The goal of this part of the dissertation was to develop a microchannel geometry that would produce a dilution series in droplets and later enforce emulsification of this series. The developed solution passively generates a dilution series and later emulsifies the series passively by step emulsification. The emulsification of droplets is free of dead droplet volumes because of a slope that leads to the emulsification module.

The behavior of the droplet in the step emulsification module was analyzed and the influence of surface tension and flow rate in the system on the parameters of the generated emulsions and on the frequency of droplet generation was elucidated. A passive solution to the problem of nozzle clogging was developed. Parallelization of the passive nozzles was also done with remarks to the interplay between nozzles and the influence of gutter flow on emulsification in a parallelized step emulsification system. Backflow of liquids during droplet breakup in step emulsification was registered directly for the first time.

This part of work is described in the chapters 3.2. and 4.2.

C. Separation, incubation, and detection of signals from emulsions

The aim of this part of the dissertation was to develop a method of generation of series of nanoliter droplet emulsions of bacterial medium with bacteria in the same concentration and with antibiotic diluted between emulsions that would allow for separation of these emulsions without removing them from the microfluidic device, for incubation of these emulsions, and for detection of fluorescent signals from each droplet from each emulsion. A novel method of separation of emulsions with a third immiscible liquid phase was used to separate emulsions and to gather them in a polyethylene tubing. This new method allowed for identification of reaction conditions with only knowing the order of generated emulsions without chemical labeling of droplets. Incubation was possible in the tubing, and the detection was done with a confocal microscope.

This part of work is described in chapters 3.3. and 4.3.

D. Screening of antibiotic resistance and the inoculum effect down to the single-cell level

The aim of this part of the dissertation was to analyze the viability of bacteria in isolated populations in the presence of an antibiotic at different concentrations with different initial number of the bacterial cells in the populations, including populations containing only single isolated cells. Each population was separated from others in droplets of bacterial medium. Dependency of the level of antibiotic resistance on the initial number of cells in the populations was measured at high throughput. Influence of antibiotic concentration on the morphology of bacterial populations was shown. A hint towards a distribution of antibiotic resistance in a population of isogenic cells was found, suggesting phenotypic heterogeneity. The assays described here would be laborious and time-consuming or non-trivial if the methods described in A, B and C were not employed.

This part of work is described in the chapters 3.4. and 4.4.